"FORMULATION DEVELOPMENT AND EVALUATION OF FIXED DOSE COMBINATION OF ANTIHYPERTENSIVE AND ANTIHYPERLIPIDIMIC IN BILAYER TABLET".

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Abstract

The basic aim of this study deals with formulation and evaluation of bilayer tablet of Antihypertensive (amlodipine) and Antihyperlipidimic (atorvastatin). The Amlodipine and atorvastatin was used in treatment of hypertension and high cholesterol. Bilayer tablet was prepared by wet granulation method. Total 9 batches were prepared and granules before compression were subjected for evaluation of flow properties. All the parameters were within limits as per Indian pharmacopeia which shows good flow properties. Drug and excipient compatibility was carried out by FTIR spectroscopy. In-vitro dissolution study was performed which shows batch B as a optimized batch because of low concentration of polymers in formulation. The developed formulation was found to be stable during the stability studies of 3 months.
Keyword- Amlodipine, Atorvastatin, Bilayer tablet, In-vitro study.

1 INTRODUCTION.

"Developing a combination of two or more active pharmaceutical ingredient in a single dosage form is known as bilayer tablet". The drug delivery system provides required amount of drug within short duration and also maintain the steady level of drug concentration. [1]

The preparation of bilayer tablet is used to provide systems for administration of drugs which are incompatible and to provide controlled release tablet preparation with surrounding multiple swelling layers. [2]

Bilayer drug delivery systems are designed to release the drug at two different rates or at two different time periods. i.e., they are either fast or slow. A fast/slow release provides an initial release of drug followed by constant rate of release over a specific period of time. This is used primarily when maximum relief is required quickly and followed by controlled release phase to avoid repeated administration. [3]

Bilayer tablet is suitable for sequential release of two drugs in combination for sustained release tablet in which one layer is immediate release as loading dose and second layer is maintenance dose. [4]

1.1 FIXED DOSE COMBINATION (FDC) PRODUCT:

Developing a new molecular entity is a lengthy, expensive and high-risk endeavor. Reformulating drugs that have been proven to be safe and effective into fixed-dose combination (FDC) products represents an essential strategy for drug development companies to realize maximal commercial returns.[8]

More than one-third of all the new drug products introduced worldwide during the last decade were fixed dose combination preparations. The trend varies from country to country. In Japan, only 10 percent of n e w products were FDCs, whereas, in European countries like Spain, it was up to 56 percent. However, such statistical data are not available for the developing countries, but there seems to be a trend towards increasing production and prescription of FDCs. [9]

FDCs are available for the treatment of various ailments ranging from nutritional deficiency to cardiovascular diseases. Some combination products may improve the quality of life for many patients. Such combinations (for example, anti-tubercular and antiretroviral combinations) are essential for many diseases. Many FDC preparations comprise vitamins, cough suppressants, anti-diarrheal, iron preparations, Antacids, analgesics and tonics. [10]

What are Fixed Dose Combinations?

A fixed dose combination refers to the combination of two or more drugs in a single pharmaceutical formulation in certain fixed doses.

According to ***WHO expert committee*** on specifications for pharmaceutical preparations (39th report, 2005) a FDC can be defined as follows:

"A combination of two or more API in a fixed ratio of doses."

This term is used generically to mean a particular combination of actives irrespective of the formulation or brand. It may be administered as single entity products given concurrently or as a finished pharmaceutical product.[11, 12]

Combination pharmaceutical products are also defined as being able to treat the same disease state, multiple disease states, or counteract the negative side-effects.

- **'Additive'** is combining two or more drugs with complementary modes of action to gain the desired therapeutic effect without the side-effects;
- **'Potentiation'** is the synergistic effect on drug A by adding a dose of drug B without a therapeutic effect;
- **'Cancellation'** is when effects of one drug are nullified by the addition of a second.

A combination product may contain pharmaceuticals, biopharmaceuticals, or nutraceuticals. They can also incorporate several, different controlled-release profiles, such as immediate and sustained.

The pharmaceutical industry is placing a greater emphasis on combination products and commercializing poorly-soluble compounds due to their limited pipelines of soluble compounds.

With the successful evolution of combination products, companies will continue to place increasing resources on developing new, innovative and beneficial combination products. Layered tablets have provided a successful approach in delivering combination products, primarily consisting of compatible, soluble compounds. Some of these layered tablets have included different release profiles for each compound in order to achieve the most beneficial release profile for each active. [13]

1.2 Introduction of Amlodipine:-

Amlodipine is dihydropyridine calcium antagonist, which inhibits the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It is used to treat hypertension, chronic stable angina, and confirmed or suspected vaso-spatic angina. Amlodipine is a medication used to lower blood pressure and prevent chest pain.

By widening blood vessels it lowers blood pressure. In angina, amlodipine increases blood flow to the heart muscle to relieve pain due to angina. It can be used either monotherapy or combination therapy for the management of hypertension or coronary artery disease. Amlodipine can be administered to adults and children 6-17 years of age.

1.3 Introduction of Atorvastatin:-

Atorvastatin, as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme (HMG - CoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, a precursor of sterols, and an early rate-limiting step in cholesterol biosynthesis. Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia. Is a white to off-white crystalline powder that is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and slightly soluble at pH 6.8 phosphate buffers and acetonitrile, lightly soluble in ethanol and freely soluble in methanol.

Atorvastatin calcium is highly susceptible to heat, moisture, a low pH environment and light. Again the amorphous form is many times unstable than its counterpart crystalline form. The intestinal permeability of atorvastatin is high at the physiologically intestinal pH (6– 6.5). However, it is reported that the absolute bioavailability of atorvastatin is 12% after a 40mg oral dose.

2 MATERIAL AND METHOD-

2.1 MATERIAL:-

Amlodipine, Atorvastatin, PVP K-30, SLS, HPMC, Microcrystalline cellulose, Magnesium stearate, Crosprovidone, Sodium bicarbonate, Mannitol and colour.

2.2 METHOD OF WET GRANULATION:-

The wet granulation method was used for preparation of tablets. All the ingredients were passed through sieve no.20. the amlodipine/ atorvastatin, MCC, PVP K, SLS, HPMC, were mixed together in mortar and pestle for 5 min. The disintegrant and stabilizers were added to it and mixed and the magnesium stearate was added as an lubricant to it. The dam mass was prepared and it was passed through sieve no .12. The granules was prepared by using following steps.

3 EXPERIMENTAL WORK

3.1 PREFORMULATION STUDY-

FTIR of Amlodipine, Atorvastatin, PVP K, SLS and HPMC: -

The infrared spectrum of Amlodipine, Atorvastatin, PVP K, SLS and HPMC was recorded by potassium bromide (KBr) pellet technique, in which the mixture of Amlodipine, Atorvastatin, PVP K, SLS and HPMC, and excess potassium bromide nearly at the ratio of 1:100 is mixed homogeneously and then placed in sample holder for analysis. The spectra were scanned over wavelength region of 4000 to 400 per cm. compressing into discs by applying a pressure of 8 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained.

Bulk Density:-

10 g of powder was placed in 50 ml of measuring cylinder. The initial volume occupied by the powder was noted without disturbing the cylinder and bulk density was calculated by the following equation.

Bulk density= Mass of drug / Bulk volume.

Tapped Density:-

10 g of powder was placed in 50 ml of measuring cylinder. The cylinder was fixed to taps i.e., approximately 100 times until the powder bed had reached the minimum level. The final volume was recorded and the tap density was calculated by the following equation.

Tapped density= Mass of drug / Tapped density

Hausner's Ratio:-

Hausner's ratio is measured by the ratio of tapped density to the bulk density.

Hausner's ratio= Tapped density / Bulk density.

Carr's index:-

It shows compressibility of powder. It is measured by bulk and tapped densities. In theory, the less compressible material is more flow able. A material having value less than 20 to 30% is defined as free flowing material. It can be calculated by using following formula-

Carr's index= Tapped density – Bulk density / Tapped density X 100.

Angle of repose:-

The angle of repose gives the flow ability of the powder. It was determined by funnel method were the funnel was adjusted above 2cm from the horizontal surface. The accurately weighed powder was taken in funnel and allowed to flow through the funnel freely on the surface. The diameter of powder cone was measured and the angle of repose was calculated by using follow8ing formula.

Angle of repose (Θ) = $\tan^{-1}$ 2h/d

3.2 FORMULATION TABLE OF BILAYER TABLET.

Table no, 1

Ingredients	A	B	C	D	E	F	G	H	I
Amlodipine	5	5	5	5	5	5	5	5	5
Atorvastatin	40	40	40	40	40	40	40	40	40
PVPK	5	2.5	5	5	10	5	2.5	10	10
SLS	2.5	5	10	5	0	5	2.5	5	0
HPMC	2.5	2.5	5	5	5	10	5	0	10
MCC	10	10	10	10	10	10	10	10	10
Sodium bicarbonate	5	10	0	10	5	5	5	10	0
Mannitol	10	10	10	10	10	10	10	10	10
Crosprovidone	10	10	10	5	10	5	10	10	5
Magnesium stearate	10	5	5	5	5	5	10	0	10
Color	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Total	100	100	100	100	100	100	100	100	100

3.3 EVALUATION OF TABLET:-

Hardness test:-

The force required to break in diametric form. Hardness of tablet is determined by using Pfizer hardness tester. Hardness should be 5-7kg/cm for bilayer tablet.

Thickness test:-

The thickness and diameter of tablet was determined by using vernier caliper . five tablet from each batch were used and average value were calculated.

Friability:-

Friability is loss of weight of tablet in the container/package, due to removal of fine particles from the surface. This in-process quality control test is performed to ensure the ability of tablets to withstand the stock during processing, handling, transportation and shipping. Friability test was carried out by using Roche friability tester. Ten tablets were placed in drum and rotated for 100 revolutions and 1% friability was determined. It should be less than 1%. The percentage friability was determined using following formula-

$$\text{Friability} = (W_i - W_f) / Wi \times 100$$

Where,

W_i= Initial weight and W_f = Final weight.

Weight variation:-

20 tablets were selected randomly from each batch and weighed individually to check for weight variation and results were compared with IP limits. This was calculated by using following formula-

%weight variation= [(individual weight- average weight)/ individual weight] x100

Out of 20 tablets, if 2 tablets deviate the limit perform test for another 10 tablets. If not more than 2 tablet deviate the limit, batch passes test.

Method for Dissolution Studies

1) At firstly prepare Phosphate buffer of pH. 6.8 upto 900mL which used as dissolution medium.
2) The in vitro release of Amlodipine and Atorvastatin 100mg tablet (bilayer) and studied by running batches using Dissolution test apparatus – USP type II. Each batch contained 6 tablets.
3) The tablets were weighted using analytical balance keeping them on butter paper. Then tablets were placed in the vessels of the dissolution apparatus containing 900 mL of Phosphate buffer ph. 6.8, using paddles at a speed of 75 rpm.

4) The temperature was maintained at 37°C during dissolution study. The temperature was measured using thermometer.
5) 5 mL sample from each vessel was withdrawn using 5 mL syringes at 5, 10, 15 and 30 minutes of interval and kept in marked volumetric flask.
6) Fresh dissolution medium (5 mL) was added to the vessels after each sample withdrawal.
7) All the samples were filtered using funnel and filter paper.
8) Absorbance of the samples was measured using UV spectrophotometer at wavelength of 239nm. Phosphate buffer ph. 6.8 was used as blank.

4 RESULTS AND DISCUSSION:-

4.1 IR Analysis:-

Amlodipine:-

The identity of amlodipine was confirmed by comparing IR spectrum of amlodipine with reported spectrum of amlodipine (Figure 1). The IR spectra which shows characteristic bands at 3298, 3155 which indicates O-H stretching, 3706 indicates N-H stretching etc. similar to literature. The observed characteristic peaks of are amlodipine detected in the Table.no, 2.

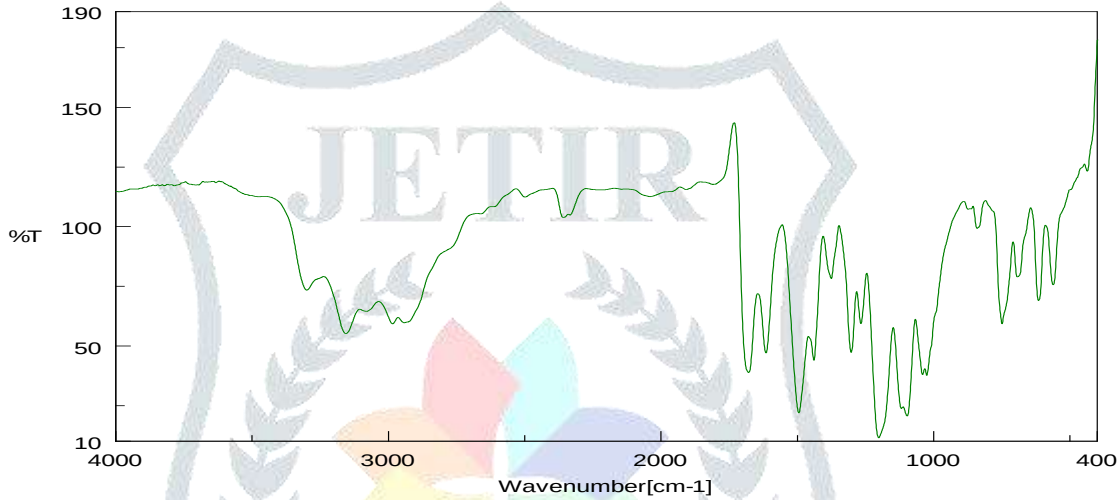

figure no: 1, ft-ir spectra of amlodipine

table no: 2, ft-ir ranges of amlodipine.

Reference	Wavenumber (cm^{-1})	Assignment
3200-3400	3298, 3155	O-H Stretching
3400-3750	3706	N-H Stretching
2900-3000	2941	Alkyl C-H Stretching
1300-1500	1494, 1375	Alkyl C-H def.
1575-1625	1615	N-H def.

Atorvastatin:-

The identity of atorvastatin was confirmed by comparing IR spectrum of atorvastatin with reported spectrum of atorvastatin (Figure 2). The IR spectra which shows characteristic bands at 3412 as O-H stretching 1352 which indicates alkyl C-H def. 1654 which indicates as C=O stretching similar to literature. The observed characteristic peaks of are atorvastatin detected in the Table 3.

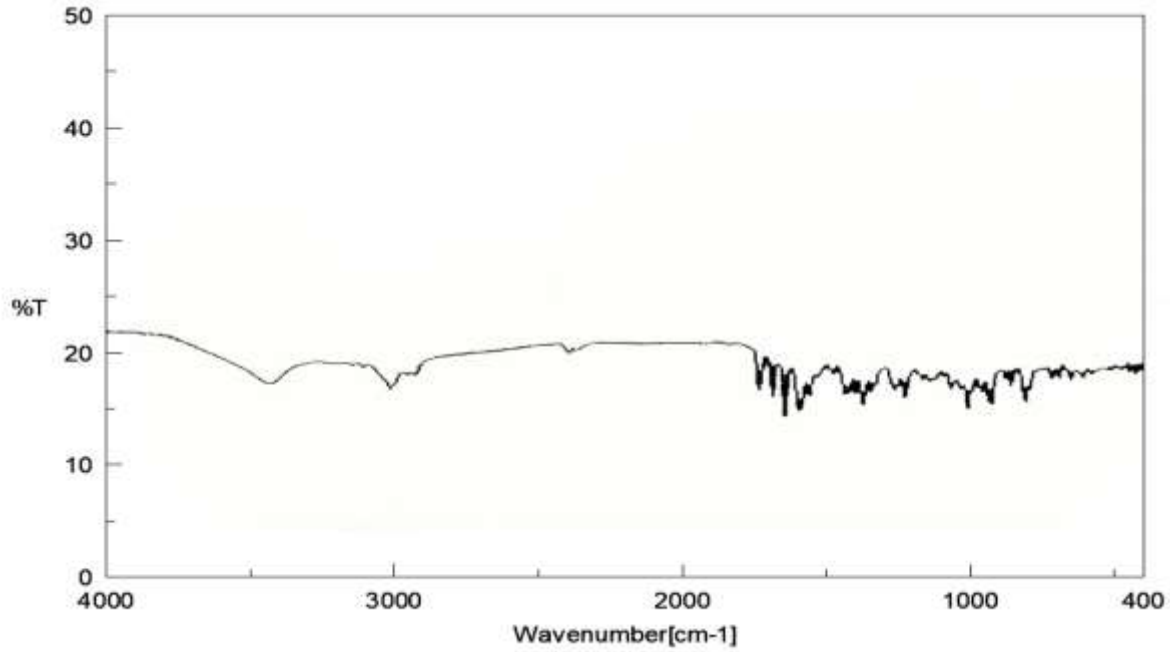

figure no: 2, ft-ir spectra of atorvastatin.

table no:3 , ft-ir ranges of atorvastatin.

Reference	Wavenumber (cm^{-1})	Assignment
3200-3400	3412	O-H Stretching
1300-1500	1352	Alkyl C-H def.
1650-1750	1654	C=O stretching
700-850	841, 756, 699	Out of plan def.

Excipient authentication:-

PVP K, SLS and HPMC were scanned by IR spectrophotometer. Each polymer shows their characteristic peak prominently as tabulated in table 4 which confirmed the purity of each polymer.

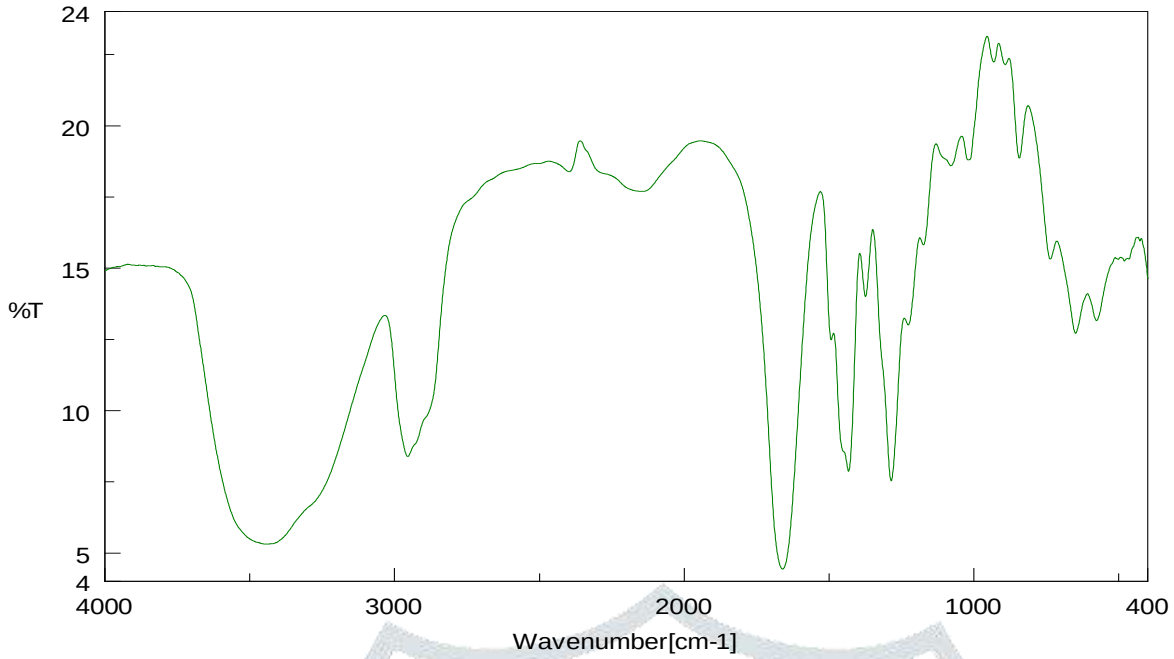

figure no: 3, ft-ir spectrum of pvp k

IR spectrum shows dominant characteristic peak of PVP k. Especially, O-H, alkyl C-H and C-O and the stretching vibrations at 3450, 2954 and 1285 respectively, which confirms the polymer was authentic one.

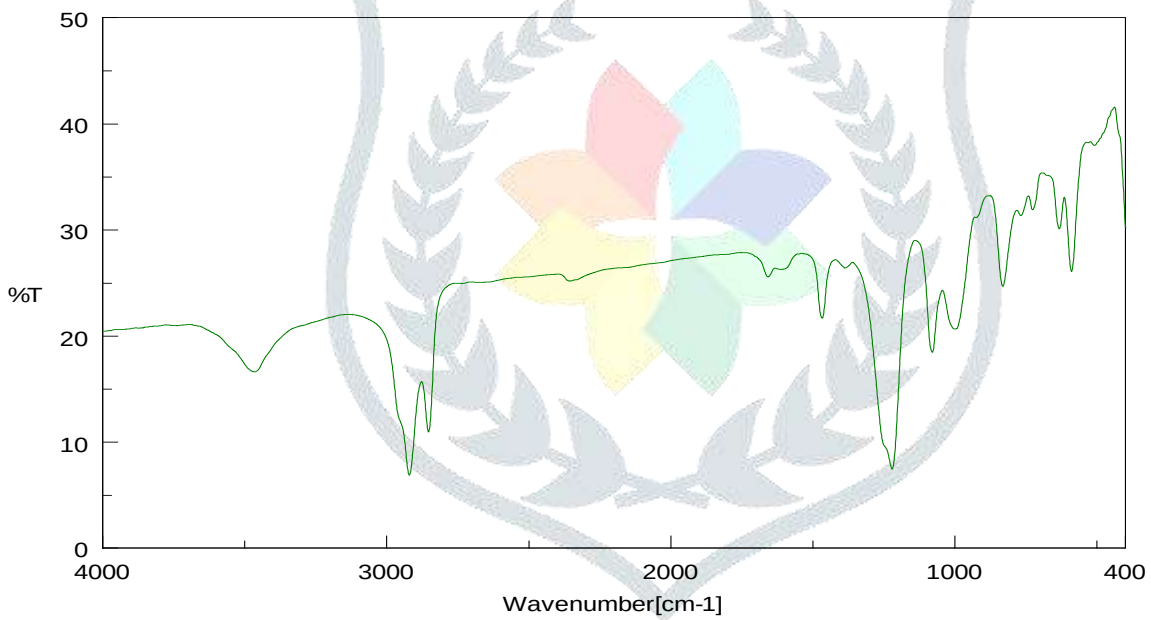

figure no: 4, ft-ir spectra of sls

IR spectrum shows dominant characteristic peak of SLS. Especially, O-H, C-H and C=C and the stretching vibrations at 3470, 2926 and 1285 respectively, which confirms the polymer was authentic one.

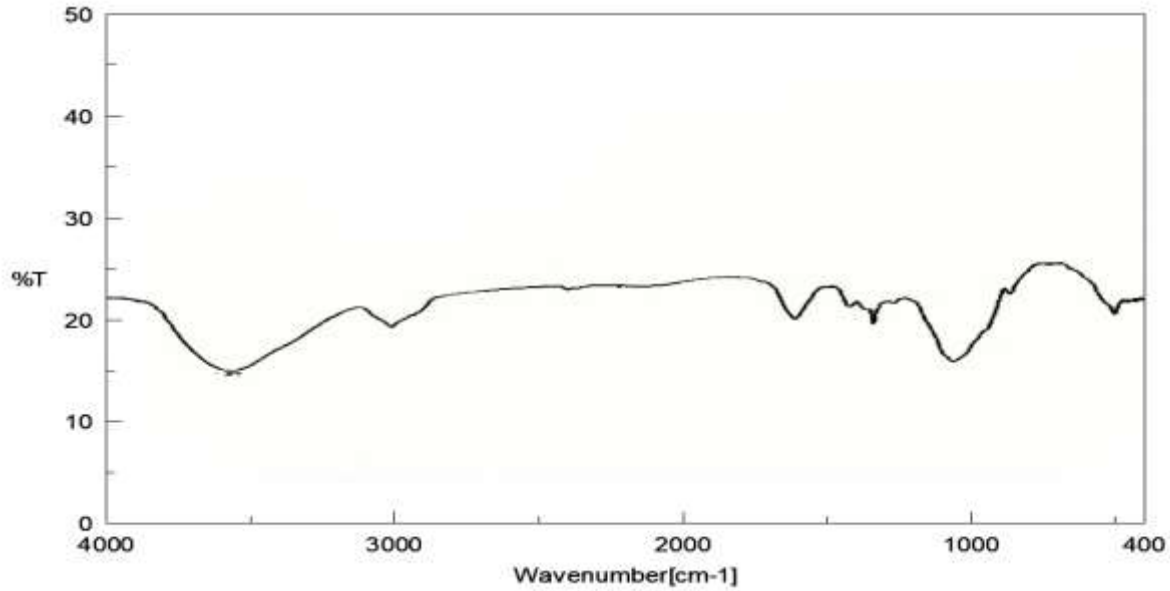

figure no: 5, ft-ir spectra of hpmc.

IR spectrum shows dominant characteristic peak of SLS. Especially, O-H, alkyl C-H and C-O and the stretching vibrations at 3465, 2853 and 1079 respectively, which confirms the polymer was authentic one.

table no: 4, ft-ir ranges of hpmc, pvp k and sls

Polymer	Wavenumber (cm^{-1})	Assignment
HPMC	3470.28	O-H Stretching
	2926.45	C-H Stretching
	1642.09	C=C Stretching
PVP K	3450	O-H
	2954	Alkyl C-H
	1431,1374	Alkyl C-H def.
	1285	C-O stretching in ether
SLS	3465	O-H stretching
	2853	Alkyl C-H stretching
	1467,1221	Alkyl C-H def.
	1079	C-O stretching in ether
	1221(m), 996(s)	C-O coupled with O-H def. in primary alcohols

4.2 EVALUATION OF GRANULES:

Preformulation studies for all pre-compression parameters were studied and the results of all pre-compression parameters for all the batches are tabulated in Table 5 and 6.

table no: 5, results of pre-compression parameters for batch a-i

	Amlodipine			Atorvastatin			Mean		
Batch no	Bulk density	Tapped density	Hausner's ratio	Bulk density	Tapped density	Hausner's ratio	Bulk density	Tapped density	Hausner's ratio
A	30	35	1.16	40	49	1.22	35	42	1.19
B	32	40	1.25	40	46	1.15	36	43	1.2
C	28	33	1.17	39	45	1.15	33	39	1.16
D	33	30	0.91	39	52	1.33	36	41	1.12
E	30	35	1.16	35	49	1.4	35	42	1.28
F	35	31	0.88	39	52	1.33	37	41.5	1.10
G	28	37	1.32	44	45	1.02	36	41	1.17
H	30	35	1.16	36	45	1.25	33	40	1.20
I	31	39	1.25	35	41	1.17	33	40	1.21

table no: 6, results of carr's index and angle of repose.

Carr's index	Flow ability	Angle of repose	Flow rate
18	Good	28.88	Good flow
15	Excellent	22.87	Excellent flow
15	Excellent	23.74	Excellent flow
11	Excellent	22.5	Excellent flow
13	Good	26.2	Good flow
11	Excellent	24.6	Excellent flow
11	Excellent	23.2	Excellent flow
17	Good	28.78	Good
16	Good	26.20	Good

Pre-compression parameters were studied and results for all pre-compression parameters such as, bulk density and tapped density of all batches was found to be in limits while the Hausner's ratio of batch B and batch F shows excellent flow property and batch A, C, D, E, G, H and I shows good flow property. The Carr's index of batch B,C,D,F and G shows excellent flow ability while batch A, E,H and I shows good flow ability and angle of repose was found to be between 22.5^{o} to 28.88^{o} within the limits which shows good flow property.

4.3 EVALUATION OF POST COMPRESSION PARAMETER:-

Formulation studies for all post compression parameters were studied and the results of all post compression parameters for all the batches are tabulated in Table 7

table no: 7, results of post-compression parameters for batch a-i.

Sr no	Hardness (kg/cm^2)	Thickness (mm)	Friability (%)	Weight variation (gm)	Disintegration time (min)
A	5	3.6	0.45	5.14	3
B	5.5	3.5	0.29	4.23	2
C	5.5	3.5	0.38	5.41	3
D	5	4.2	0.20	2.61	5
E	5.5	4.8	0.26	3.97	2
F	5	4.8	0.33	5.41	4
G	5	5.4	0.23	3.32	5
H	5.6	3.4	0.40	3.98	5
I	5	3.6	0.46	5.33	3

The limits for hardness are 5-7kg/cm2, and all the batches showed hardness within a limit. Friability of all batches was below 1% which was also within a limit. Weight variation is less than ±5% which compiles with official limit, and disintegration time was found to be in limits. The results of post compression parameters for all 9 batches of bilayered tablet were comply with official limits.

4.4 IN-VITRO DRUG RELEASE:

table no: 8, in-vitro % cumulative drug release profile of batch a-i.

Time in min.	A %CDR	B %CDR	C %CDR	D % CDR	E% CDR	F % CDR	G % CDR	H % CDR	I % CDR
0	0	0	0	0	0	0	0	0	0
10	2.076428	7.195611	0.851305	0.98562	3.2654	0.2618	1.1264	2.3108	5.2491
20	4.888569	9.539917	1.007946	1.2315	7.25684	0.6138	2.3518	3.952	11.6249
30	5.975066	32.23231	1.819902	1.9851	13.2568	1.2951	3.0218	5.06813	17.6816
40	9.541909	55.37571	2.116534	2.3658	19.26587	9.6257	4.001	6.9283	23.6819
50	15.29259	64.79001	2.145289	3.2548	24.26589	13.2671	4.9283	8.9273	31.81671
60	17.89673	68.25047	2.184639	5.6981	37.2659	19.9892	5.82049	12.2861	38.9016
70	24.82814	79.88271	2.208854	7.3259	42.16587	26.5937	6.892	15.3704	45.2933
80	30.85767		2.474461	8.2145	50.32181	32.5671	8.9204	19.3182	53.2719
90	35.13561		2.773364	9.86512	59.3654	43.2649	9.987	23.281	61.9212
100	43.53777		2.720393	11.2658	65.2681	48.6243	11.2961	28.6192	69.0543
110	53.83572		3.291714	16.5942	71.3568	58.951	12.6509	34.1927	76.2043
120	59.2028		3.58759						
130	62.35668		4.220961						
140	72.06886		1.533298						
150	78.22109		9.931896						
160	96.27488								

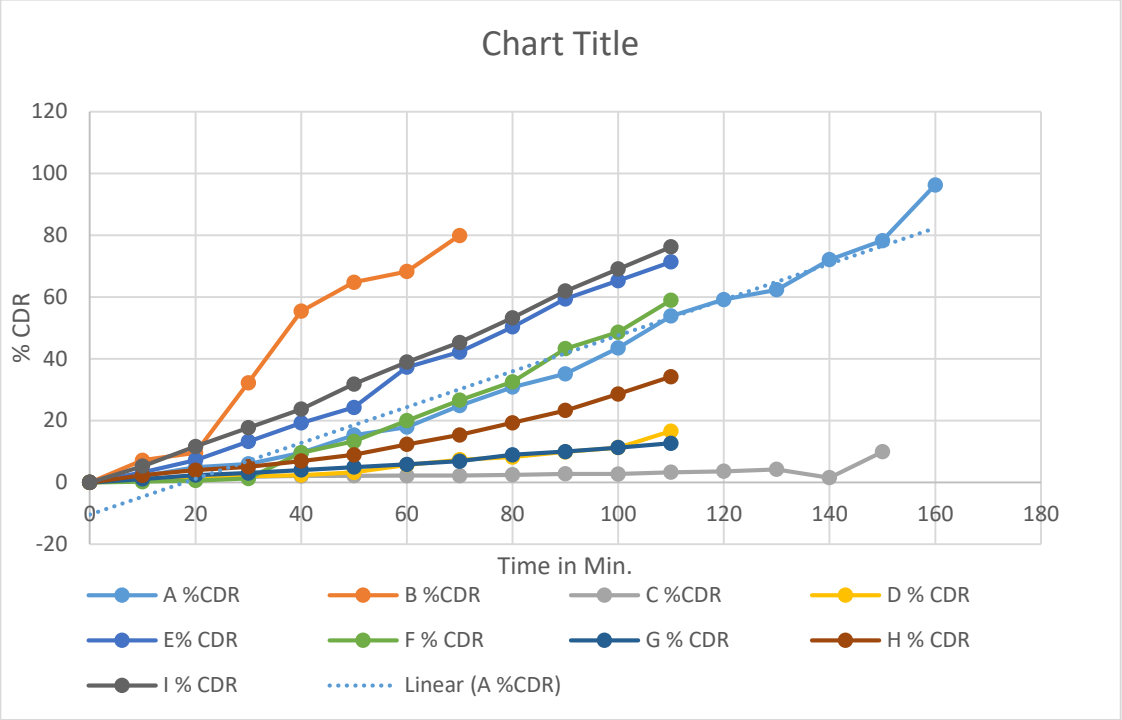

figure no: 6, in-vitro % cumulative drug release profile of bilayer tablet.

From the dissolution study it is concluded that release of drug is largely dependent on polymer. The drug release study was carried out up to 3 hrs. The percentage drug release from batch A to I vary from 9.93 to 96.27%. Batch B release 79.88% of drug within 70 min as compare to batch E and I. The percentage drug release of other batches D, E, F, G, H and I at second hour varies from 16.59% to 76.20% while batch A shows 96.27% drug release but the time taken for it was 3 hrs. While the batch C shows very poor drug release 9.93% at 3hrs. Finally it had concluded that batch B is optimized batch because the low concentration of polymers such as PVP k (2.5), SLS (5) and HPMC (2.5) which gives higher drug release in less time than other batches.

5 CONCLUSION:-

The present work was formulation and evaluation of bilayer table of amlodipine and atorvastatin. The investigation aim of the present work was to develop the bilayer tablet of amlodipine and atorvastatin to provide once a daily dose administration of amlodipine and to maintain the drug release time of atorvastatin for prolonged duration of 3 hours and to maintain therapeutic effect.

The bilayer tablet provides one of the important design approaches where two drugs were completely separated with different release rates and can be incorporated in a single unit. In the present investigation the bilayer tablet formulation development by taking some trial batches with different polymer concentrations.

Bilayer tablet technology was adopted which was having two separate layers for both drugs. Wet granulation method was used for preparation of bilayer tablet

In the present work bilayer tablet had one layer was sustained release layer of atorvastatin and another was immediate release layer of amlodipine. The polymer used in different concentrations was HPMC, PVP K and SLS. The Crosprovidone was used as superdisintegrant

Characterization of drugs was done by performing determination of melting point, UV and FTIR. From the FTIR spectra analysis, it was concluded that there was no interaction between the drugs and polymers as the principle peaks of the drugs were found unaltered in the spectra of drug, polymer, and physical mixture.

Bilayer tablets were evaluated for in vitro dissolution, friability, thickness, hardness, weight variation, disintegration test and powders like Angle of repose, Bulk density, Tapped density, Carr's index and Hausner's ratio was done.

The concentration of polymers was optimized by varying the concentration from 2-10% and best results were obtained with B batch which showed 55.37% drug release at 40 min and 79.88% drug release at 70 min which was used for further study in optimization of bilayer tablets.

Hence, the optimizing batch was found to be with medium level of HPMC, PVP K and SLS that is batch B

The formulation was optimized on the basis of the % drug release, thus the B batch was selected as an optimized formulation because it gave the best results in vitro dissolution 79.88% drug release for 70 min.

The given formulation showed spontaneous release of amlodipine ad atorvastatin at the 1 hour followed by percentage drug release for 3 hours which was the main objective of present investigation and that was achieved.

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